

Images and Pixels

- For our purposes an image is a grid of pixels locations
- Each pixel has a single brightness value
- Pixels are arranged on a rectilinear grid in rows and columns
- Image segmentation is the process of identifying the objects in an image
- Segmentation is a key step in extracting information from an image

Brightness, Contrast, Normalization

0.5	0.5	0.5	0.5	0.5
0.5	0.7	0.7	0.7	0.7
0.5	0.7	0.80	0.80	0.80
0.5	0.7	0.80	0.90	0.80
0.5	0.6	0.6	0.6	0.6

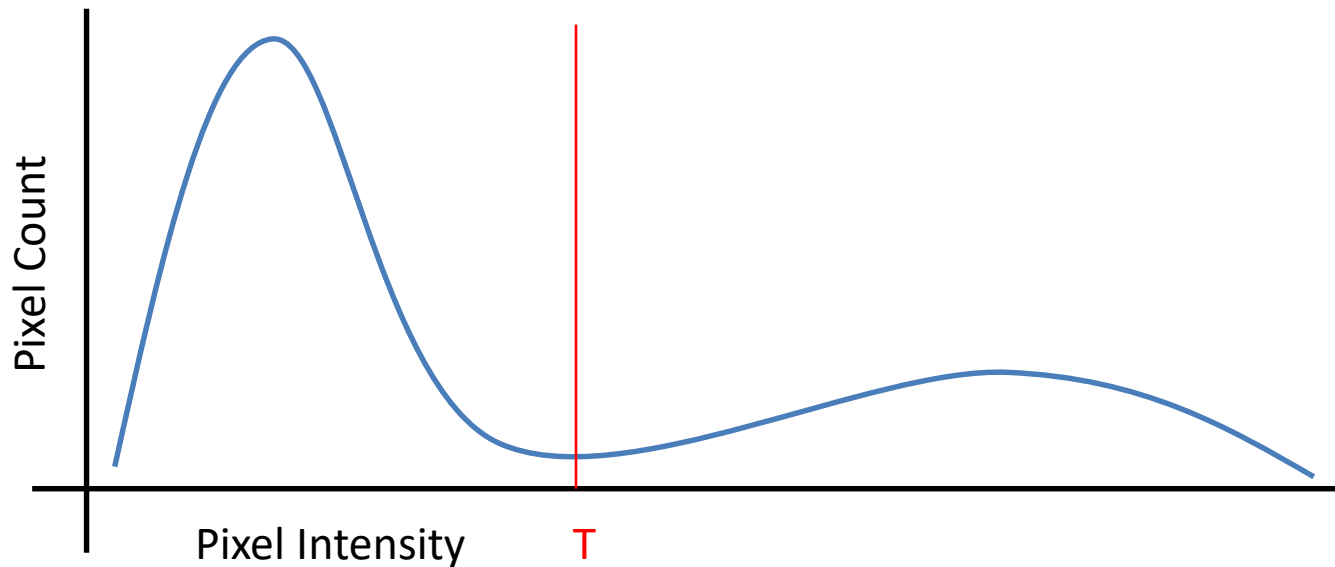
Matlab Example: dimeight.tif and speight.tif

Segmentation by Intensity Thresholding

0.5	0.5	0.5	0.5	0.5
0.5	0.7	0.7	0.7	0.7
0.5	0.7	0.80	0.80	0.80
0.5	0.7	0.80	0.90	0.80
0.5	0.6	0.6	0.6	0.6

Segmentation by Intensity Thresholding

Calculating a threshold

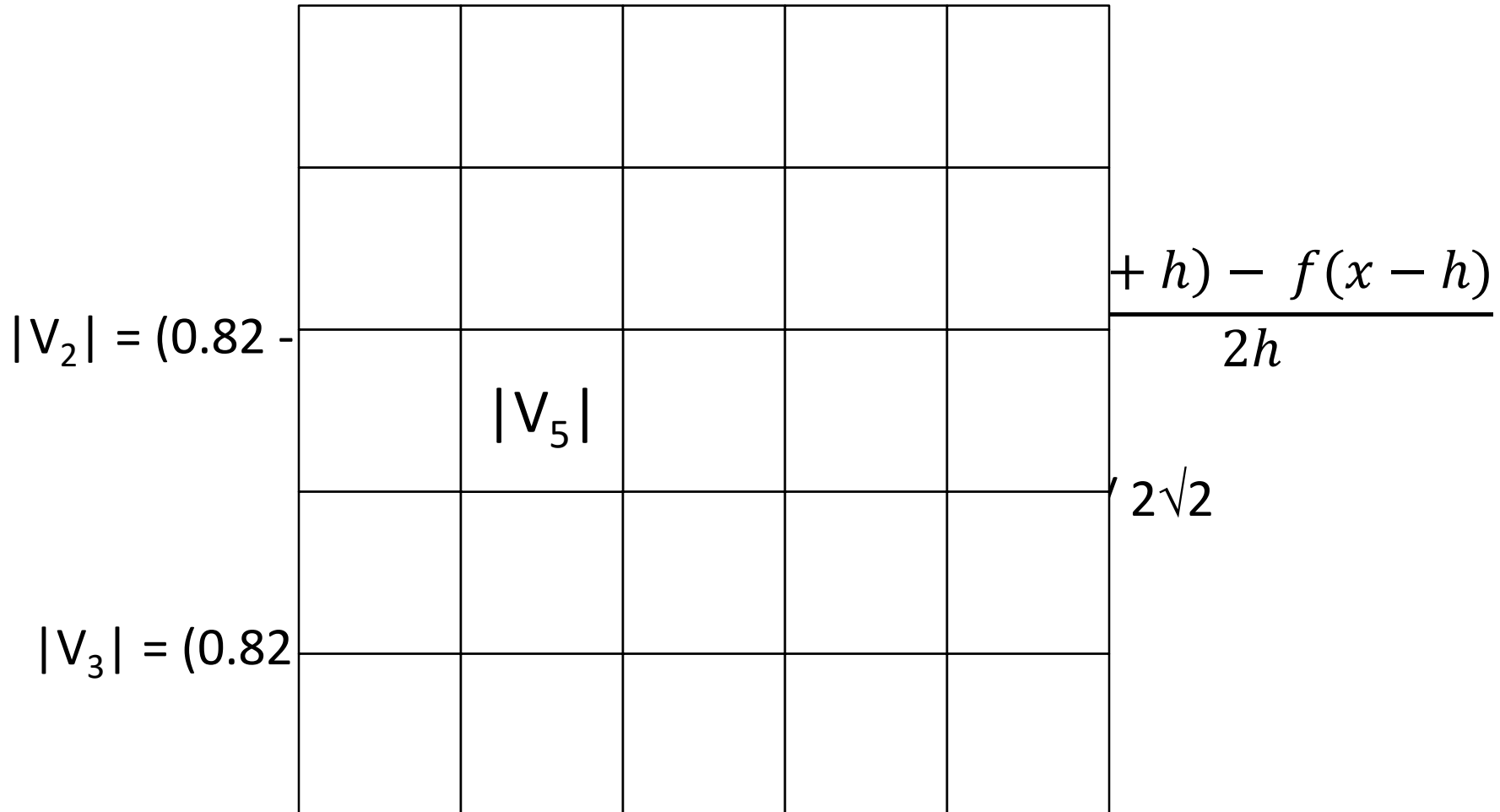


A threshold value divides pixels into two classes: foreground objects and background

Otsu's method finds a threshold that minimizes the weighted sum of the variance of the two classes

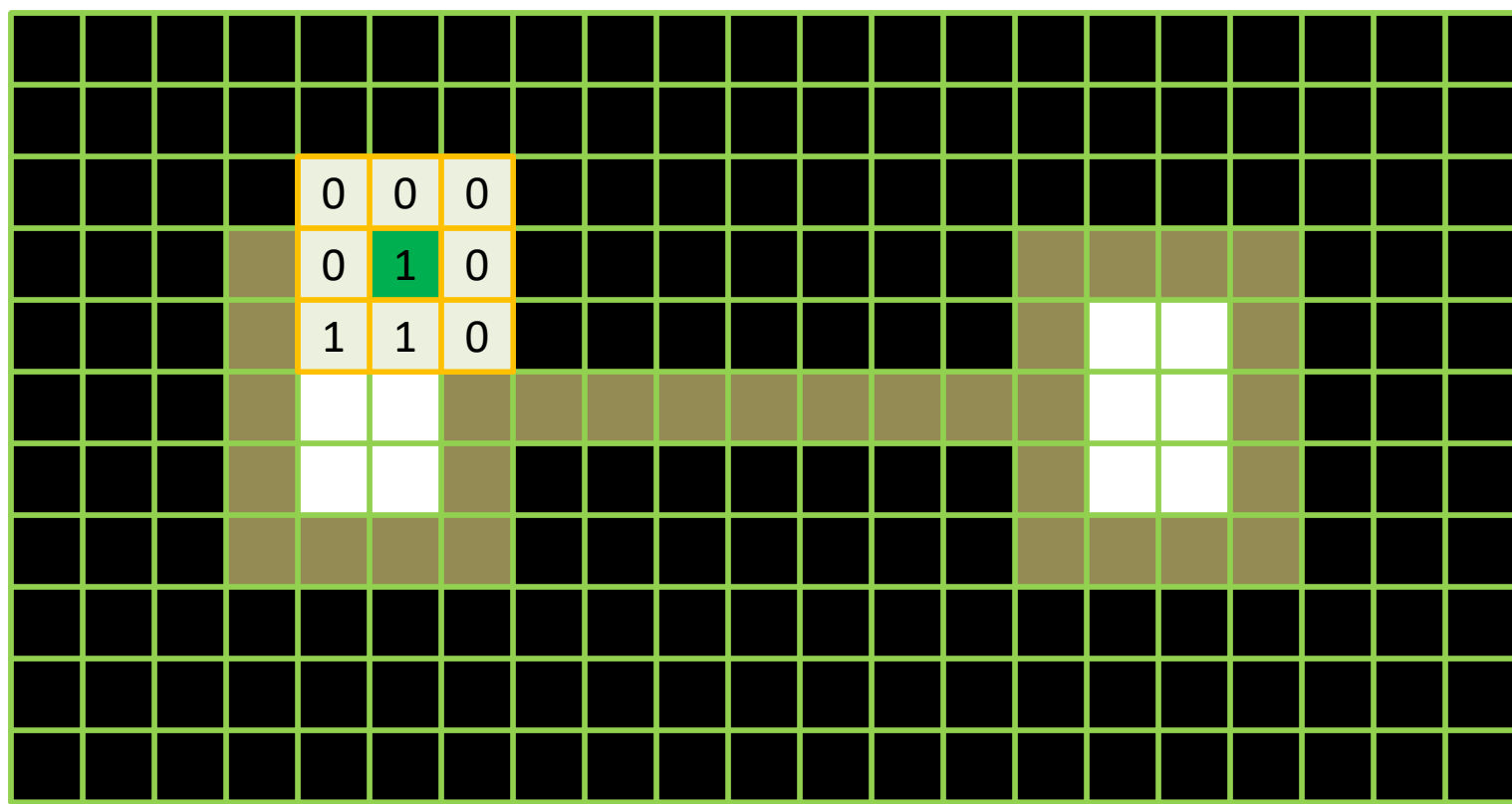
Matlab Example

Segmentation by Edge Detection

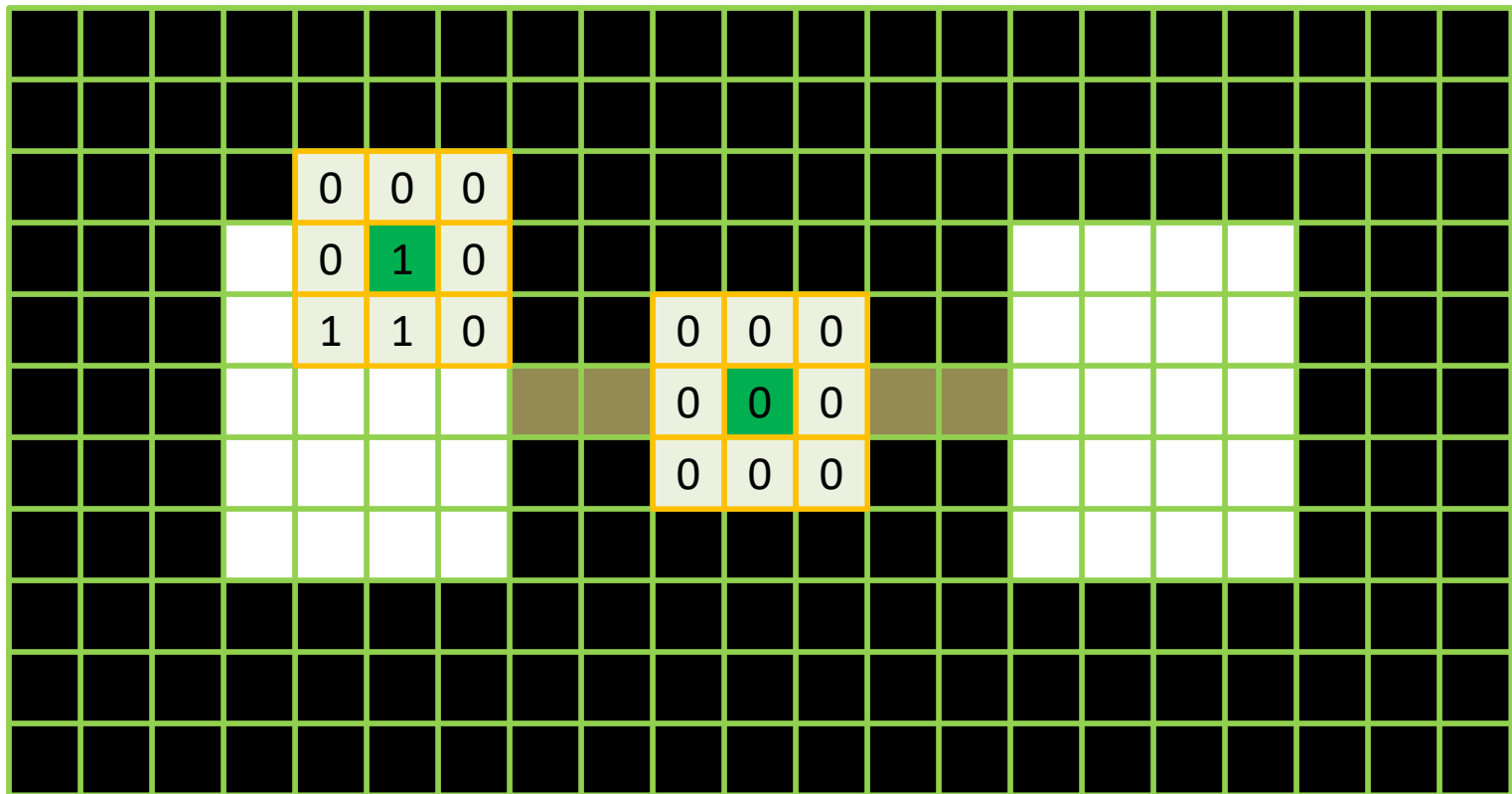


Matlab Example

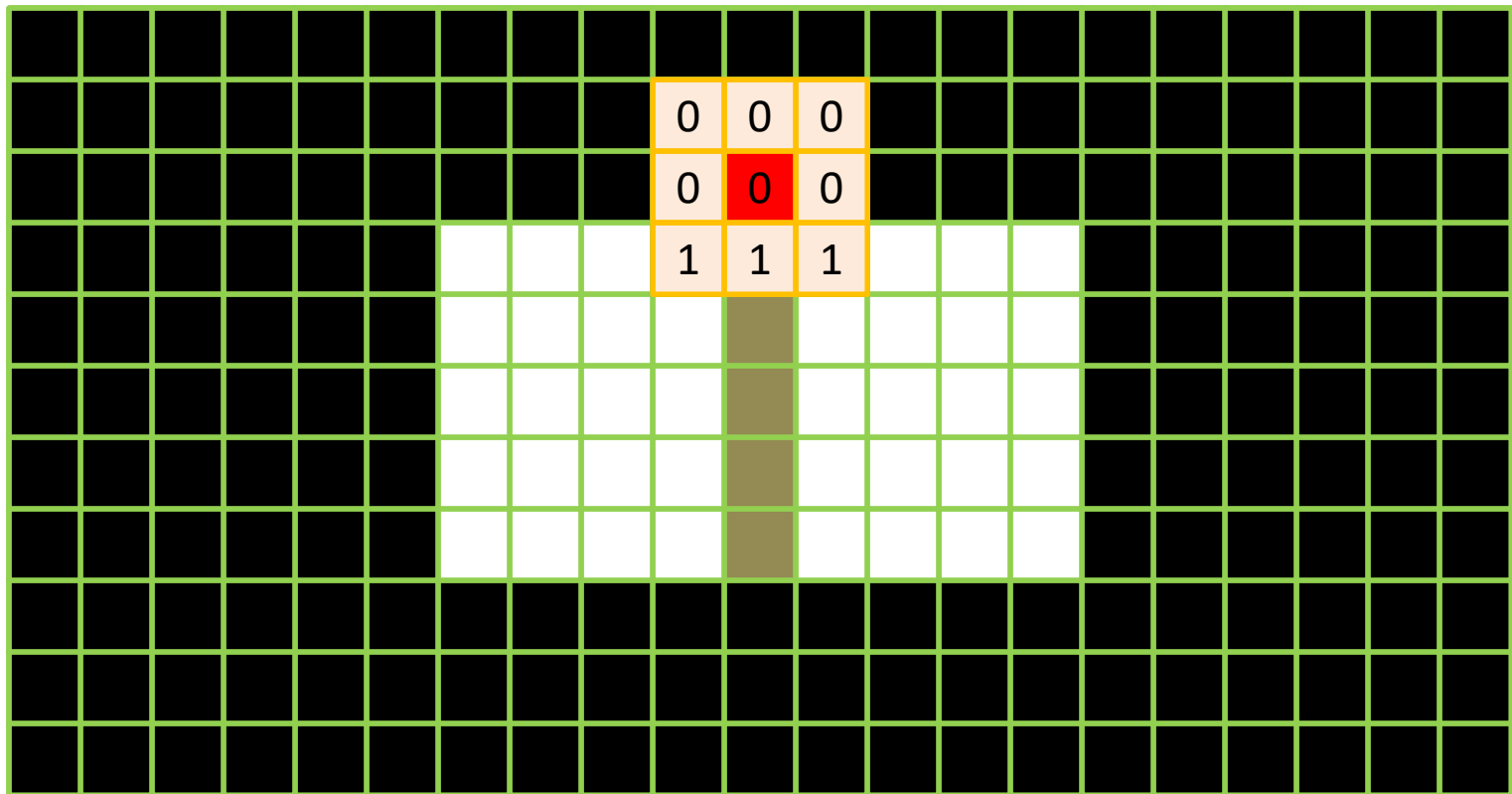
Morphological Erosion / Dilation



Morphological Erosion / Dilation



Morphological Dilation / Erosion



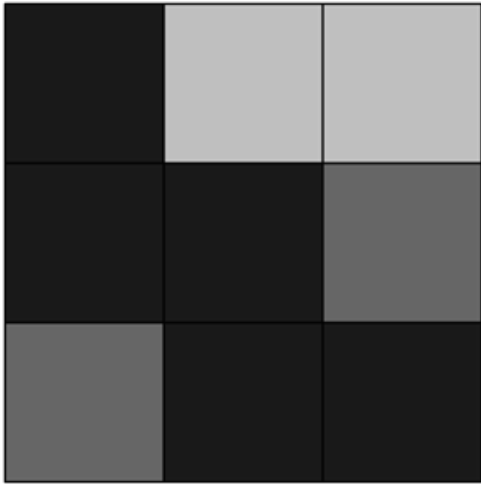
Matlab Example

Segmentation by Watershed



Segmentation by Watershed

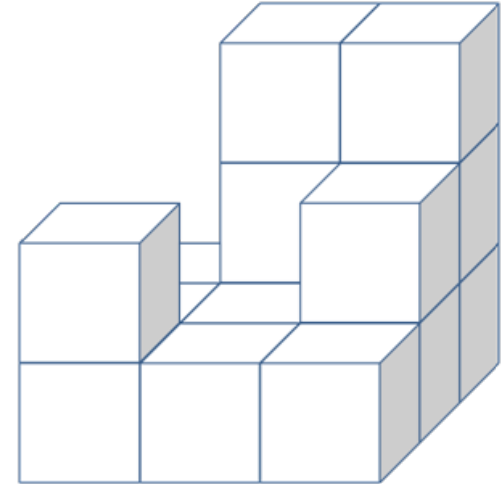
a



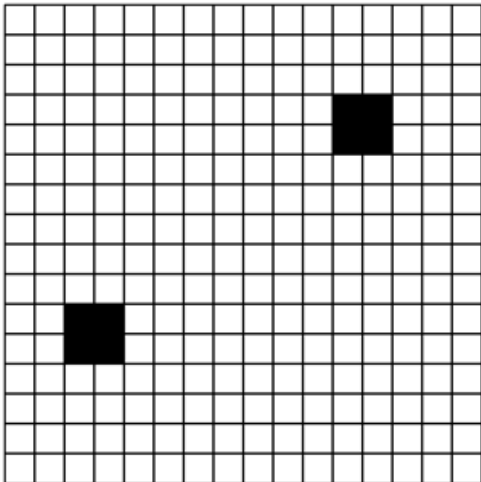
b

.2	.6	.6
.2	.2	.4
.4	.2	.2

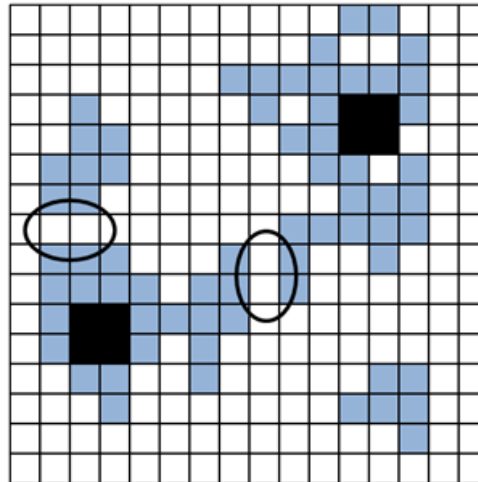
c



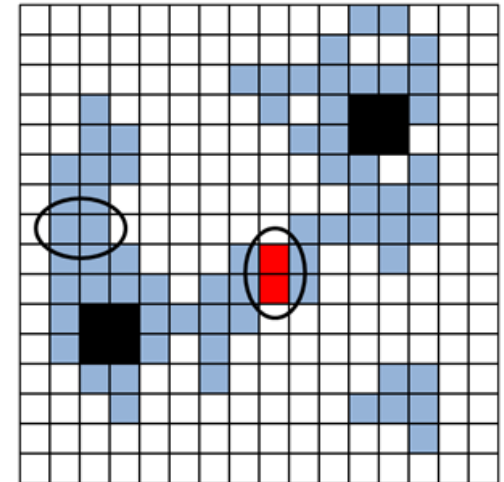
d



e

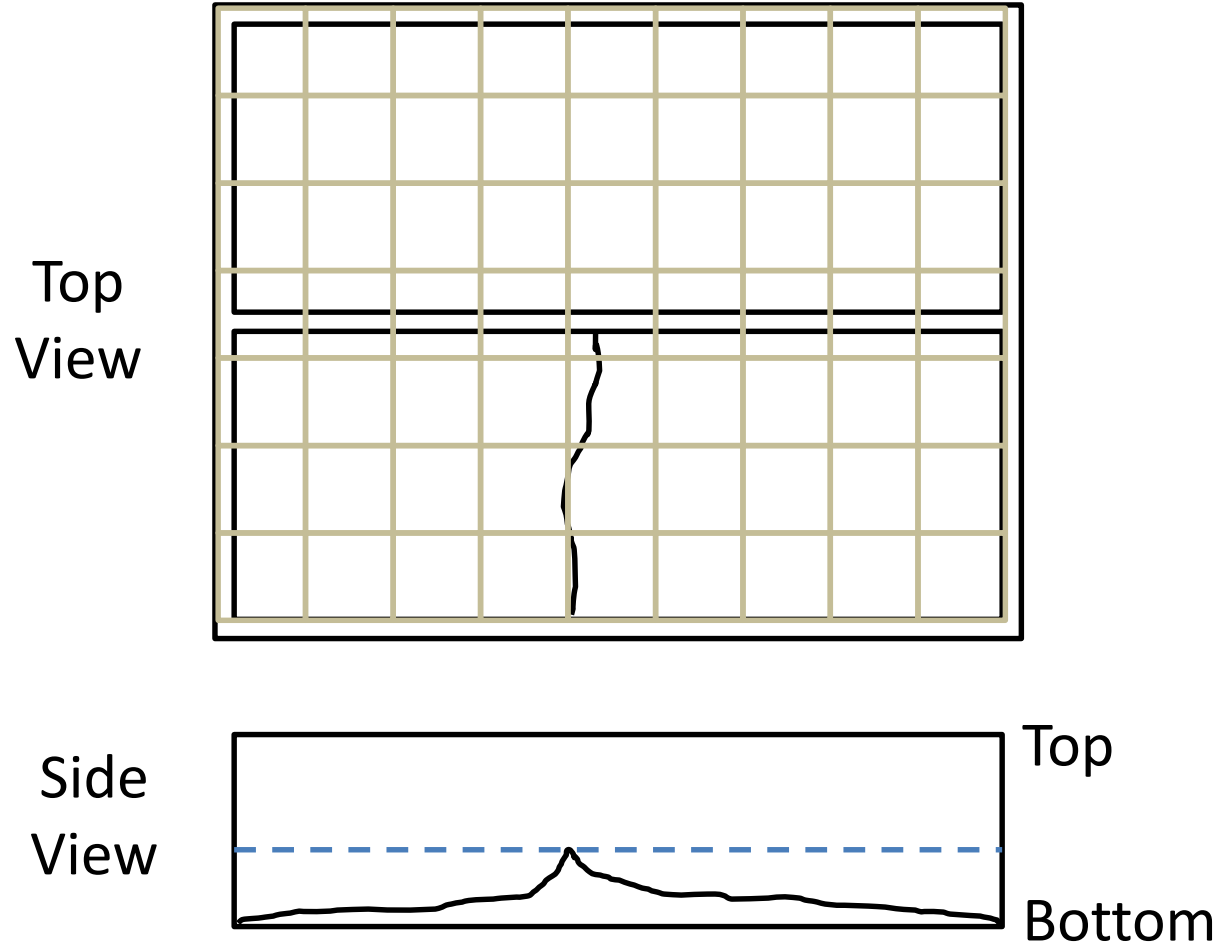


f



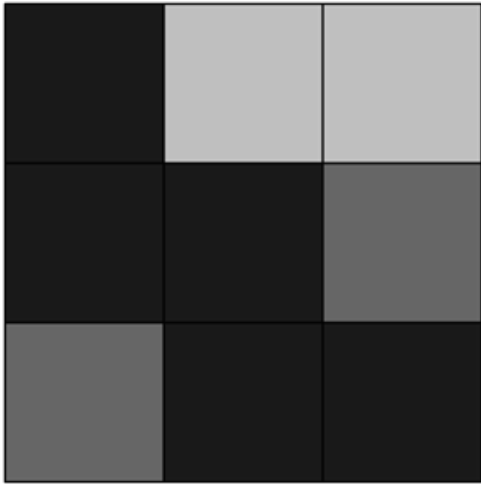
Segmentation by Watershed

Oversegmentation



Segmentation by Watershed

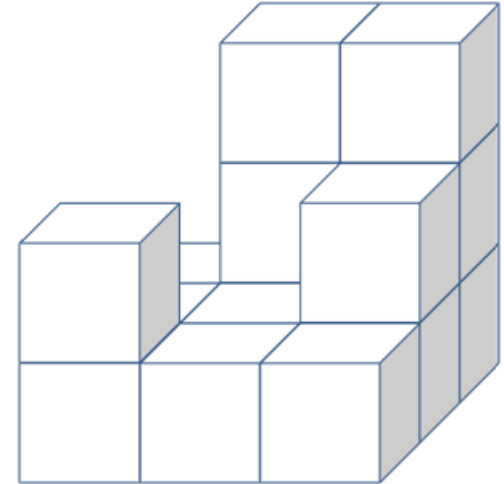
a



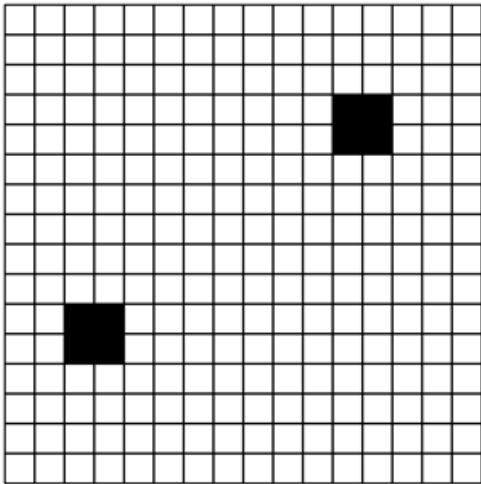
b

.2	.6	.6
.2	.2	.4
.4	.2	.2

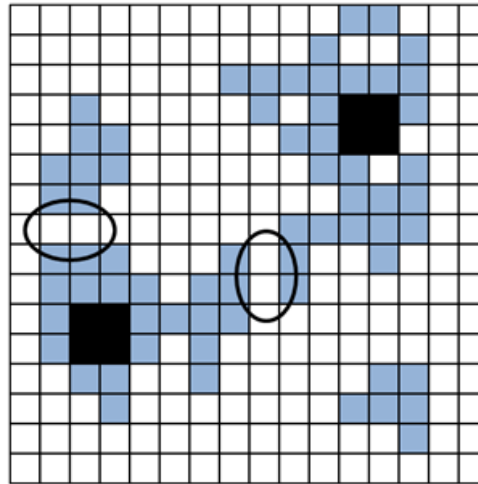
c



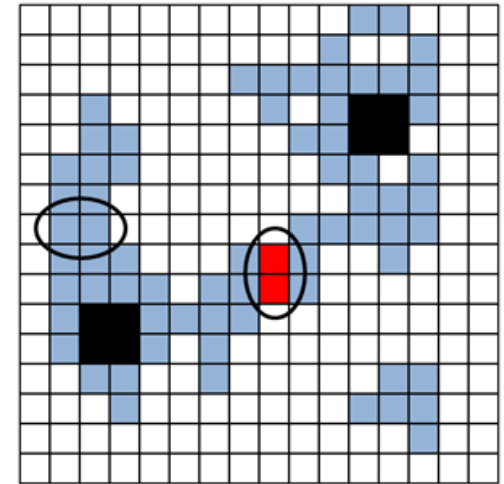
d



e



f



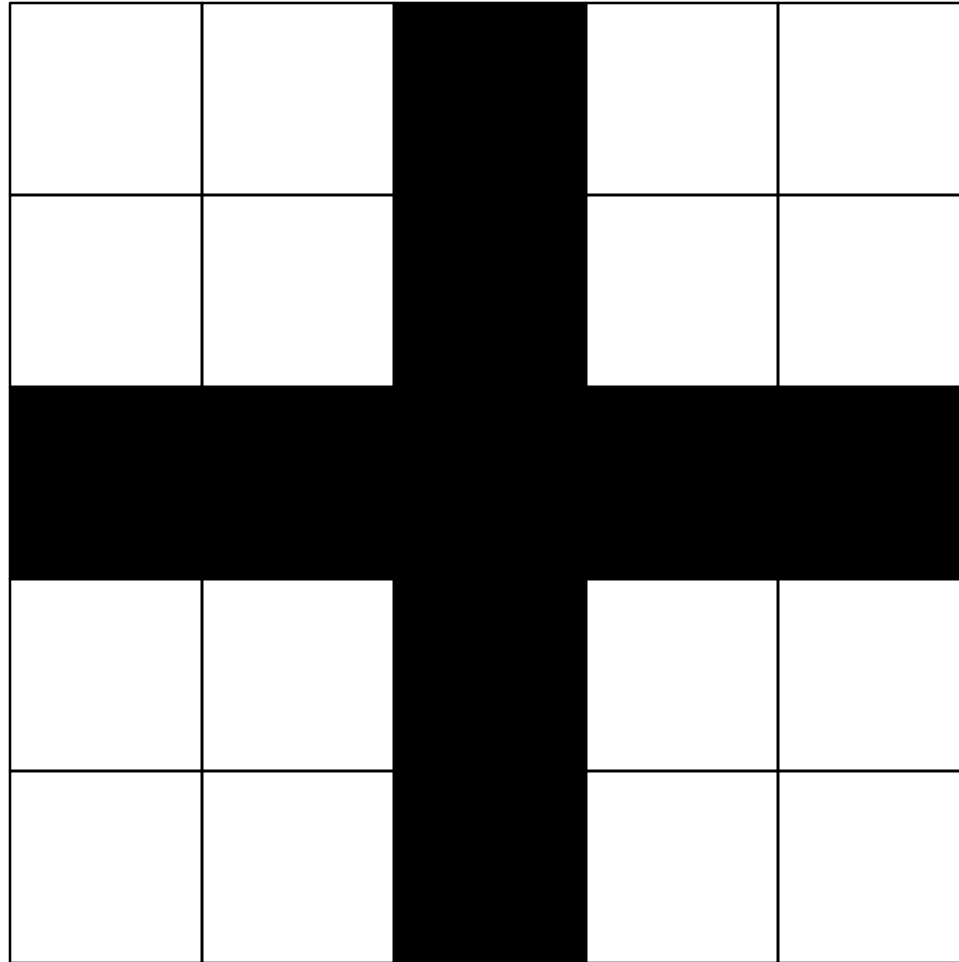
Segmentation by Watershed

In Matlab:

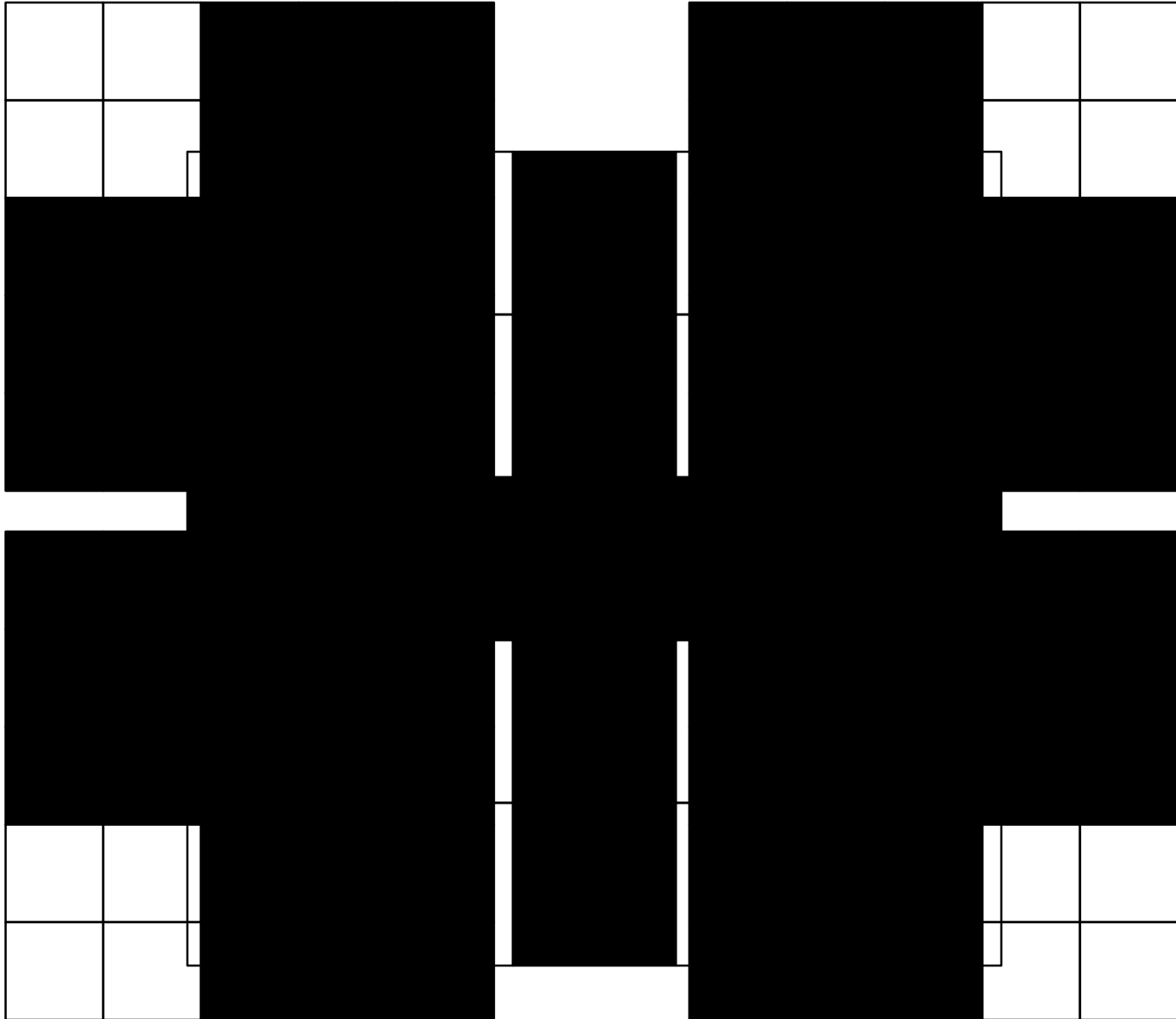
1. Compute a mask that has a marker for each foreground object.
2. Compute a marker for the background
3. Invert the image, making bright pixels dark and dark pixels bright. The goal is to make borders high (bright)
4. Use the markers from steps 1 and 2 to create minimums in the image. This prevents oversegmentation.
5. Run the watershed function
6. The watershed result treats the background as if it were a foreground object. Remove it leaving only the desired foreground.

Matlab Example

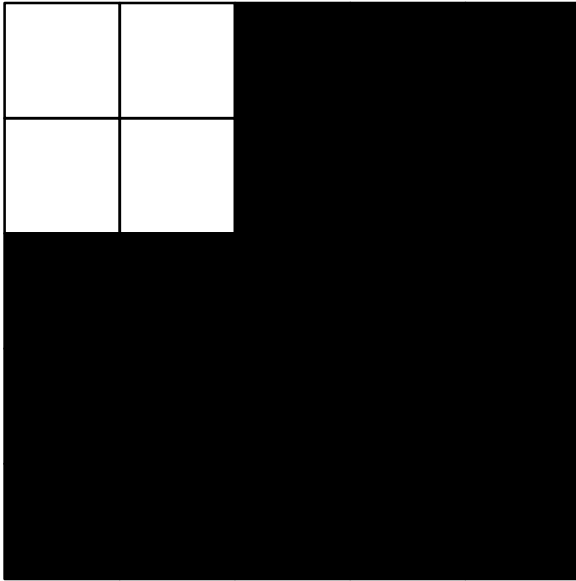
Using Masks to Extract Data



Using Masks to Extract Data



Using Masks to Extract Data



Use the mask directly for area, center, perimeter, etc.

35	23	8	61	12
27	41	55	9	81
15	16	17	18	19
33	3	0	77	44
9	2	30	22	7

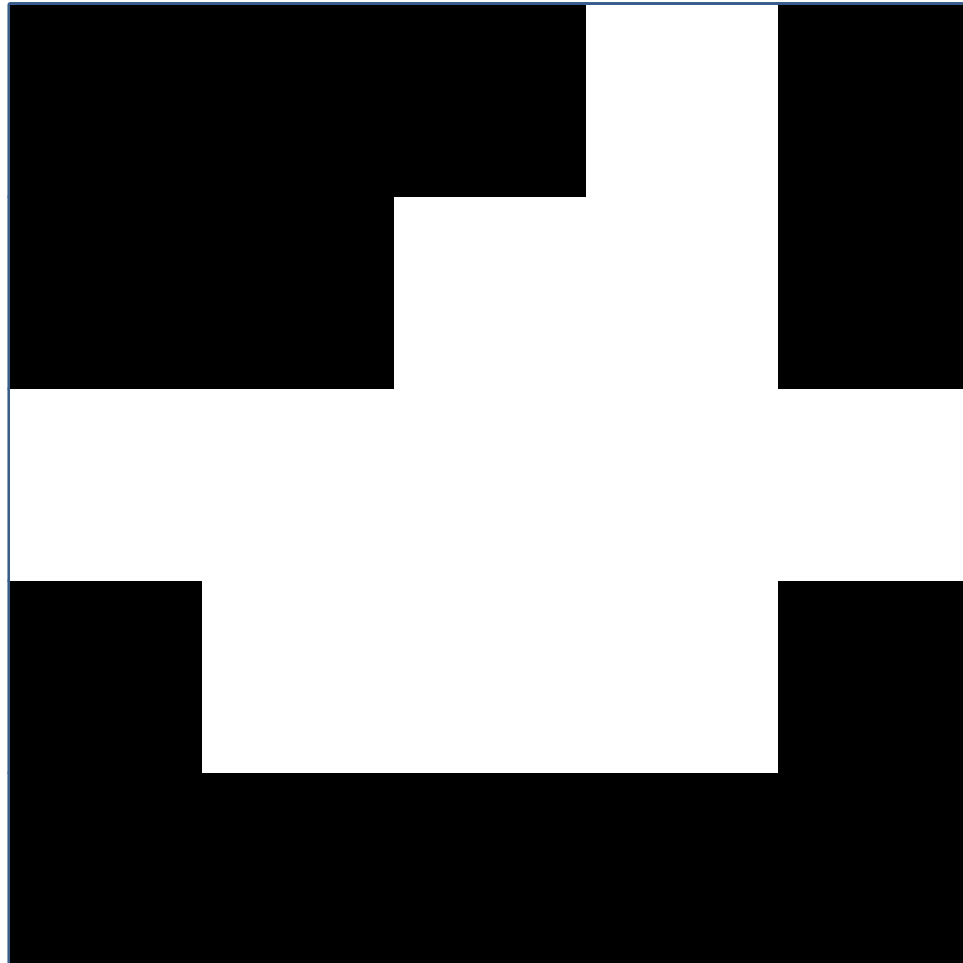
Use the mask to locate stain values inside the cell for average, standard dev., etc.

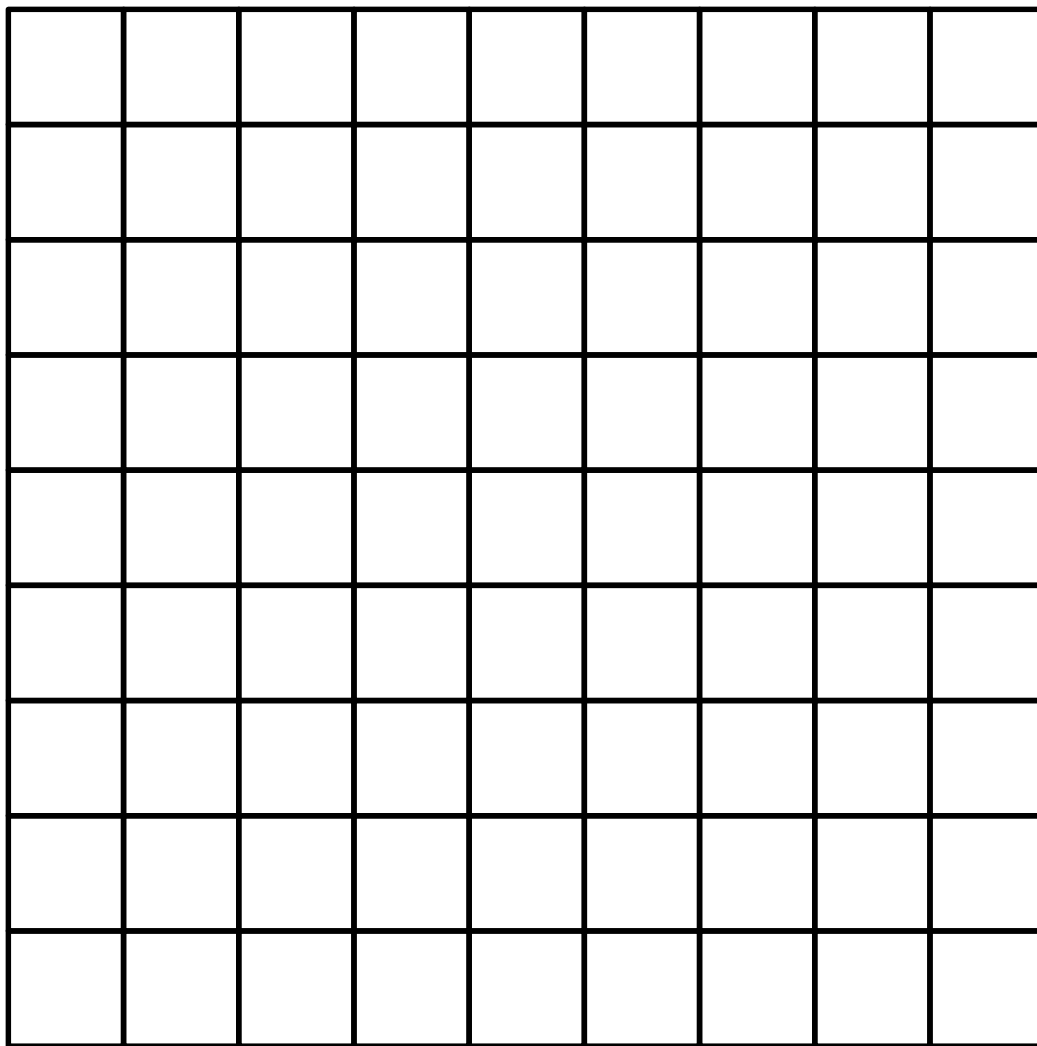
Using Masks to Extract Data

Matlab Example

	$ V_5 $			

Segmentation by Intensity Thresholding





What is the largest number that can be expressed in n bits?

